Defective platelet α-granule biogenesis, thrombo-inflammation and wound-healing in Nbeal2-deficient mice

Supplementary Information

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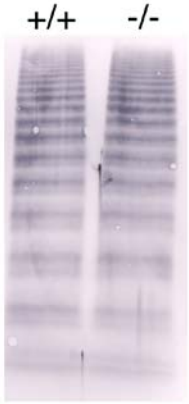
Supplemental Table 1

Expression levels of major glycoproteins of resting wild-type (+/+) and *Nbeal2*$^{-/-}$ (-/-) platelets and after stimulation with 0.5 μg/ml convulxin and 0.1 U/ml thrombin. Results are expressed as MFI ± SD (n=4 mice per group) and are representative of 4 independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. n.s. indicates not significant.

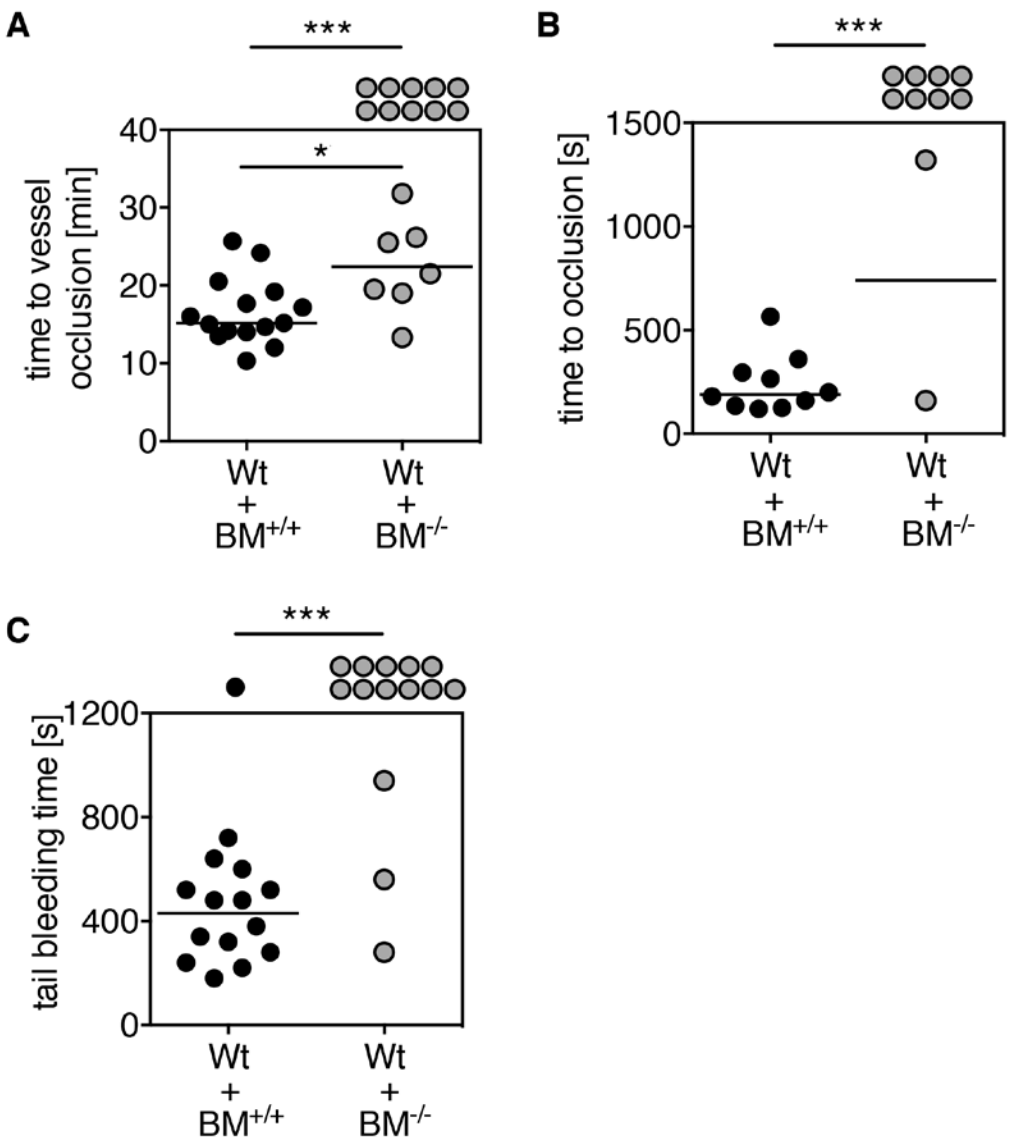
	Resting			**CVX**			**Thr**		
MFI	**+/+**	**-/-**	**Sign.**	**+/+**	**-/-**	**Sign.**	**+/+**	**-/-**	**Sign.**
GPIb	325 ± 9	365 ± 4	**	274 ± 21	255 ± 29	n.s.	196 ± 8	226 ± 33	n.s.
GPV	261 ± 9	268 ± 5	n.s.	266 ± 15	191 ± 16	***	34 ± 3	29 ± 2	*
GPIX	362 ± 9	410 ± 11	***	382 ± 20	392 ± 20	n.s.	383 ± 30	389 ± 23	n.s.
CD9	1149 ± 18	1226 ± 44	*	1515 ± 96	1595 ± 76	n.s.	1787 ± 122	1636 ± 66	*
GPVI	43 ± 1	42 ± 2	n.s.	36 ± 2	35 ± 3	n.s.	57 ± 3	47 ± 4	**
α2	47 ± 3	46 ± 1	n.s.	67 ± 7	65 ± 8	n.s.	75 ± 8	57 ± 10	*
β1	140 ± 12	135 ± 4	n.s.	169 ± 11	155 ± 7	*	177 ± 13	158 ± 8	*
αIIbβ3	552 ± 38	632 ± 37	**	680 ± 62	596 ± 53	n.s.	801 ± 80	652 ± 61	*
CLEC-2	112 ± 8	123 ± 8	n.s.	183 ± 27	166 ± 11	n.s.	211 ± 21	163 ± 13	**

SUPPLEMENTAL FIGURES



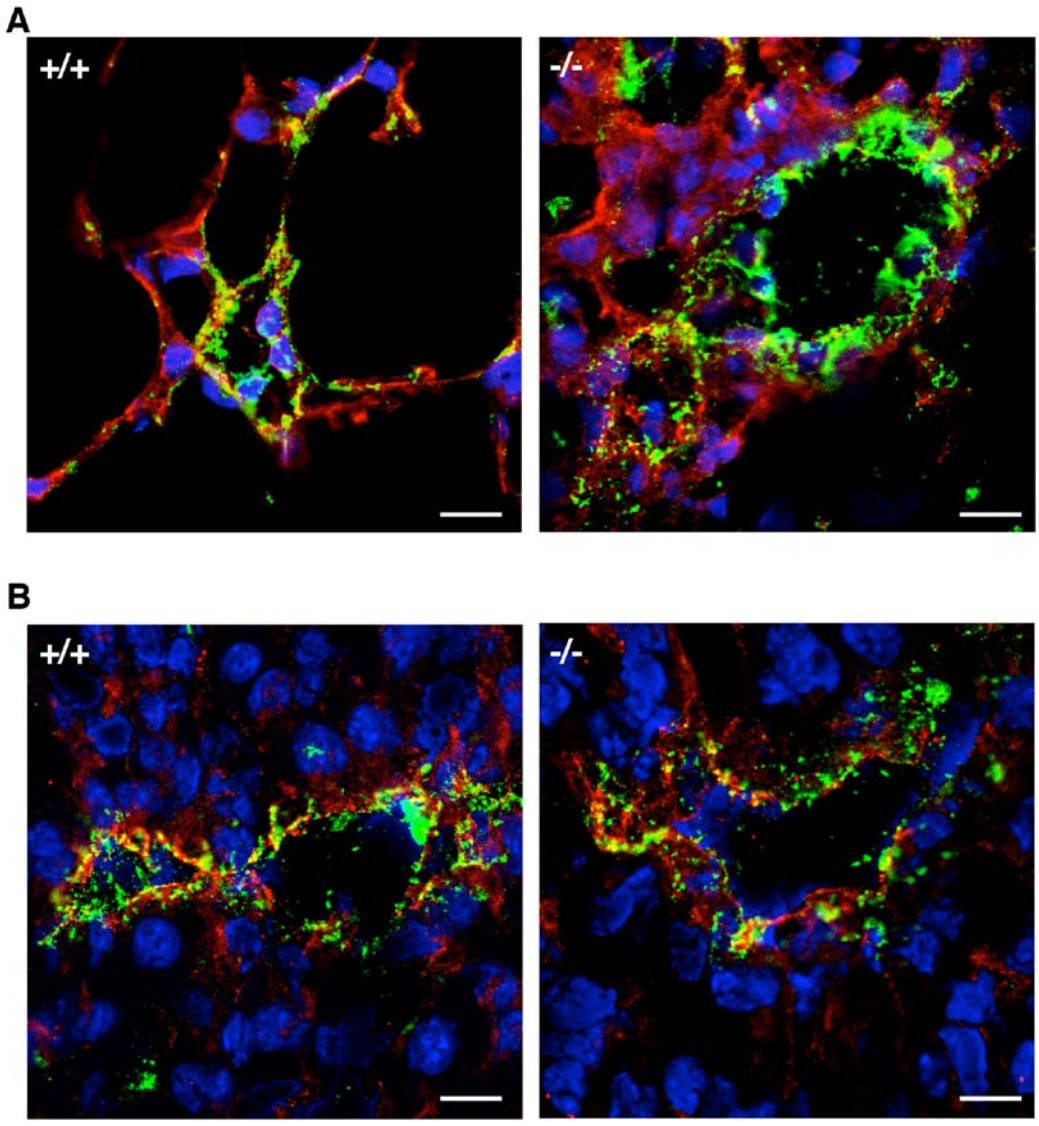
Supplemental Figure 1

vWF multimer distribution of wild-type (+/+) and knockout (-/-) mice was analyzed in citrated whole blood. Note the triplet structure, equal distribution and presence of high molecular weight multimers in both samples.



Supplemental Figure 2

Severely impaired arterial thrombus formation and hemostasis in irradiated wild-type mice reconstituted with *Nbeal2*$^{-/-}$ bone marrow. (A) Thrombus formation in small mesenteric arterioles was induced by topical application of 20% $FeCl_3$. Time to stable vessel occlusion is depicted. Each symbol represents one arteriole. (B) In an aorta injury model the blood flow was monitored for 30 min or until complete occlusion occurred. (C) Tail bleeding times of wild-type and *Nbeal2*$^{-/-}$ mice. (B, C) Each symbol represents one individual. *, $p < 0.05$; ***, $p < 0.001$.



Supplemental Figure 3

Normal vWF distribution in endothelia of wild-type (+/+) and knockout (-/-) mice. Cryo-sections of (A) lung and (B) liver were stained for vWF (green) and endothelia using endoglin (red) as an endothelial marker. Cell nuclei were stained using DAPI (blue). Bars 10 mm.

SUPPLEMENTAL METHODS

vWF multimer analysis. Citrated plasma was analyzed in the central laboratory of the University Hospital, Würzburg. In short, vWF multimers were separated by electrophoresis with SDS 2.25% agarose gel. The same amount of vWF antigen was loaded for each sample. Gels were blotted on nitrocellulose membrane and multimers were detected using rabbit anti-human-vWF (DAKO) and donkey anti-rabbit secondary antibody coupled to alkaline phosphatase (Dianova). BCIP/NBT (Sigma) was used to visualize the multimers directly on the membrane.

Cryo sectioning and staining. Fresh tissues were embedded in Tissue-Tek (Sakura) and flash-frozen swimming on liquid nitrogen. 5 μm sections were cut and stored at -20°C. Sections were stained using anti-CD105 (anti-endoglin, BioLegend) antibody which was labeled with Alexa 647 labeling kit (Invitrogen) and rabbit anti-human-vWF antibody (DAKO) plus goat anti-rabbit Alexa 488 (Invitrogen) secondary antibody and visualized using a Leica TCS SP5 confocal microscope (Leica Microsystems).